# WEST Search History

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| ☐ | L8 | HIV with Tat with protein with (mutations or mutants) and position near ("27" or "51" or "55" or "79") | 10 |
| ☐ | L7 | L1 and guillon.in. | 2 |
| ☐ | L6 | HIV near Tat with protein with (mutation or mutant) and position near ("27" or "51" or "55" or "79") | 0 |
| ☐ | L5 | L1 and position near ("27" or "51" or "55" or "79") | 12 |
| ☐ | L4 | L1 same position near ("27" or "51" or "55" or "79") | 0 |
| ☐ | L3 | L1 and "Cys27Ser" | 0 |
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| ☐ | L1 | HIV same Tat same protein same (mutation or mutant) | 644 |

END OF SEARCH HISTORY

polyomavirus T antigens, SV40 VP1, and the *Xenopus laevis* $N_1/N_2$ and nucleoplasmin proteins (Kalderon *et al.*, 1984; Kleinschmidt *et al.*, 1986; Richardson *et al.*, 1986; Wychowski *et al.*, 1986; Burglin and De Robertis, 1987).

To determine the importance of each of these regions in *tat* activation, oligonucleotide-directed mutagenesis of the *tat* gene was performed. Point mutations were made which changed the first cysteine of each of the four Cys-X-X-Cys motifs to serine residues. Additional mutations were constructed which replaced several of the lysine or arginine residues in the basic region of the gene with the acidic amino acid glutamic acid. Mutations which altered both the proline residues in the N terminus of *tat*, and truncated *tat* proteins with alterations in the positions of the N and C termini were also constructed. These results indicate that the cysteine-rich and basic domains were both required for full transcriptional induction by the *tat* protein. In addition, truncated forms of *tat* did not function well if either the N terminus was displaced or the C terminus was moved upstream into the basic domain suggesting that those regions contain information required for optimal function of the *tat* protein.

## Results

### *Oligonucleotide-directed mutagenesis of the tatI protein*

Figure 1 shows the structure of the first 72 amino acids in the second exon of the *tat* gene and the amino acid substitutions introduced by oligonucleotide mutagenesis. This exon was cloned into the M13 vector, mp19, and subjected to oligonucleotide-directed mutagenesis. Mutants which replaced both the second and third prolines or the third and fourth prolines in the N terminus of the protein were constructed as were mutants in which the first cysteine of each of the four potential zinc fingers was replaced with serine residues (Figure 1). A conservative change was also made in this latter region whereby an asparagine residue was changed to a threonine residue. In the basic region at the carboxy end of this exon, mutations were made which substituted glutamic acid residues for lysine or arginine residues (Figure 1). A conservative substitution in this domain was also made by changing an asparagine to a threonine. An additional construct was made which destroyed the initiating methionine for *art*, but retained the *tat*-protein-coding sequence. Finally, truncations of the N terminus of the *tat* protein were constructed in which the initiating methionine in *tat* was moved downstream while another series of mutations introduced stop codons at various locations in the C terminus of the protein. These mutants were then cloned downstream from the Rous sarcoma virus promoter in an expression vector containing the SV40 splice acceptor and polyadenylation signals and tested for their ability to transactivate the HIV LTR (Garcia *et al.*, 1987).

### *Single amino acid substitutions alter tat activation of the HIV LTR*

Each of the mutant *tat* constructs or a control expression plasmid (RSV-$\beta$-globin) were transfected into HeLa cells together with a construct containing a portion of the HIV LTR from $-177$ to $+83$ fused to the chloramphenicol acetyltransferase (CAT) gene. Each set of transfections was repeated five times with similar results in each experiment.

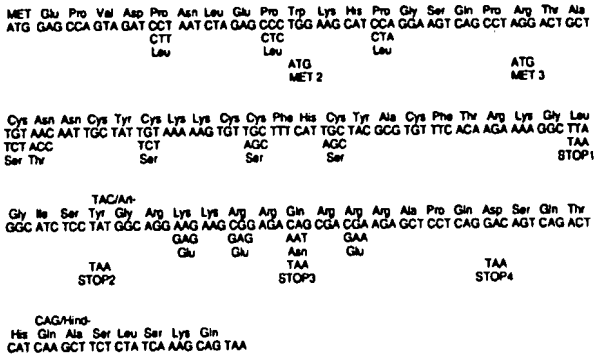


**Fig. 1.** HIV *tat* amino acid sequence and oligonucleotide-directed mutants. The first 72 amino acids in the HIV *tat* protein and the substitutions introduced by oligonucleotide-directed mutagenesis are indicated.

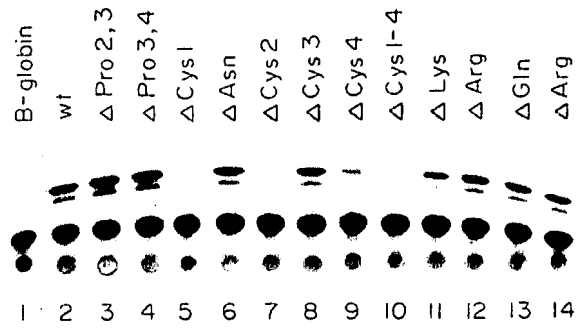


**Fig. 2.** CAT assays of HIV *tat* point mutations. HIV *tat* constructs $\beta$ globin (1), wt (2), Δ Pro 2, 3 (3), Δ Pro 3, 4 (4), Δ Cys 1 (5), Δ Asn (6), Δ Cys 2 (7), Δ Cys 3 (8), Δ Cys 4 (9), Δ Cys 1–4 (10), Δ Lys (11), Δ Arg 1 (12), Δ Gln (13) and Δ Arg 2 (14) were transfected into HeLa cells with the HIV LTR CAT construct, harvested at 48 h post-transfection, and CAT activity determined.

CAT assays were performed to assay for the effects of these mutations on *tat*-induced transcriptional activation of the HIV LTR (Gorman *et al.*, 1982). Mutations of either the second and third or third and fourth proline residues in the N terminus of the *tat* protein had minimal effects on *tat* activation of the HIV LTR (Figure 2, lanes 3 and 4). Mutations of three of the four Cys-X-X-Cys motifs (Figure 2, lanes 5, 7 and 9) resulted in a marked decrease in *tat*-induced transactivation as compared with wild-type *tat* (Figure 2, lane 2). Mutagenesis of the third cysteine motif resulted in a minimal change in *tat*-induced CAT activity (Figure 2, lane 8). A mutation of all four cysteine motifs also resulted in a marked decrease in *tat*-induced activity (Figure 2, lane 10). A conservative mutation of the amino acid asparagine in this same region did not affect the trans-activating ability of the *tat* protein (Figure 2, lane 6). Mutations which substituted the acidic amino acid glutamic acid for several of the lysine or arginine residues in the basic domain of the *tat* protein resulted in a decrease in *tat*-induced activity, but not as severe as seen with the mutations of the cysteine-rich domain (Figure 2, lanes 11, 12 and 14). A conservative mutation in this basic domain that replaced an asparagine with a glutamine residue had minimal effects on *tat* transactivation (Figure 2, lane 13). Thus, at least two domains of the *tat* protein appear important for *tat*-mediated transactivation of the HIV LTR.